TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P67214US0
		US APPLICATION NO (if known, see 37 CFR 1.5) 09/926442
INTERNATIONAL APPLICATION NO. PCT/EP00/03913	INTERNATIONAL FILING DATE 2 May 2000	PRIORITY DATE CLAIMED 3 May 1999

TITLE OF INVENTION

METHODS OF DIAGNOSING OR TREATING ALZHEIMER'S DISEASE ON BASIS OF INCREASED CEREBROSPINAL FLUID LEVELS OF NERVE GROWTH FACTOR

APPLICANT(S) FOR DO/EO/US

Roger NITSCH, Christoph HOCK -and- Uwe OTTEN

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ■ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ■ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ■ Other items or information:
    International Search Report – EPO
    PCT/IB/301 Form
    PCT/IB/304 Form
    PCT/IB/308 Form
    First Page of Publication
    International Preliminary Examination Report – with Annexes

US APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
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	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) .. $710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a)-(2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .. $740.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$1040.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$890.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ☒ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**26** - 20 =	-6-	x $18.00	$ 108.00	
Independent Claims	**7** - 3 =	-4-	x $84.00	$ 336.00	
Multiple Dependent Claim(s) (if applicable)			+ $280.00	$	
			TOTAL OF ABOVE CALCULATIONS =	$ 1464.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
			SUBTOTAL =	$ 1464.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
			TOTAL NATIONAL FEE =	$ 1464.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
			TOTAL FEES ENCLOSED =	$ 1464.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 1464.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

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By _____
William E. Player
Reg. No. 31,409

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Roger NITSCH et al

Serial No.: New

Filing Date: November 2, 2001

For: METHODS OF DIAGNOSING OR TREATING ALZHEIMER'S DISEASE ON BASIS OF INCREASED CEREBROSPINAL FLUID LEVELS OF NERVE GROWTH FACTOR

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend claims 3-4, 6-11, 17 and 20-21 as follows:

3. (amended) Use of the method according to claims 1 for evaluating a treatment for Alzheimer's disease.

4. (amended) The method according to claim 1, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. (amended) The method according to claim 1, wherein said subject is a human.

7. (amended) The method according to claim 1, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

8. (amended) The method according to claim 1, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

9. (amended) The method according to claim 1, wherein said subject receives a treatment prior to one or more of said sample gatherings.

10. (amended) The method according to claim 1, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

11. (amended) The method according to claim 1, further comprising:

determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;

wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. (amended) The kit according to claim 14 further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease, wherein a varied level or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status,

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

20. (amended) The kit according to claim 14 for use in monitoring a progression of Alzheimer's disease in a subject.

21. (amended) The kit according to claim 14 for use in monitoring the success or failure of a therapeutic treatment of a subject.

REMARKS

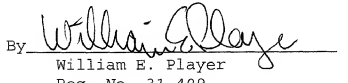
The foregoing Preliminary Amendment is requested in order to delete the multiple dependent claims and avoid paying the multiple dependent claims fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Early action on the merits is respectfully requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By 

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Atty. Docket: P67214US0
Date: November 5, 2001
WEP:jrc

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

3. (amended) Use of the method according to claims 1 ~~or 2~~ for evaluating a treatment for Alzheimer's disease.

4. (amended) The method according to claim 1 ~~any of claims 1 to 3~~, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. (amended) The method according to claim 1 ~~any of claims 1 to 5~~, wherein said subject is a human.

7. (amended) The method according to claim 1 ~~any of claims 1 to 6~~, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

8. (amended) The method according to claim 1 ~~any of claims 1 to 7~~, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

9. (amended) The method according to claim 1 ~~any of claims 1 to 8~~, wherein said subject receives a treatment prior to one or more of said sample gatherings.

10. (amended) The method according to claim 1 ~~any of claims 1 to 9~~, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

11. (amended) The method according to claim 1 ~~any of claims 1 to 10~~, further comprising:

determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;

wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. (amended) The kit according to claim 14 ~~any of claims 14 to 16~~ further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease, wherein a varied level or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status,

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

20. (amended) The kit according to claim 14 ~~any of claims 14 to 19~~ for use in monitoring a progression of Alzheimer's disease in a subject.

21. (amended) The kit according to claim 14 ~~any of claims 14 to 19~~ for use in monitoring the success or failure of a therapeutic treatment of a subject.

S/PRTS

001164wo Me/kk

03. Mai 2000

Methods of diagnosing or treating Alzheimer's disease on basis of increased cerebrospinal fluid levels of nerve growth factor

Alzheimer's disease (AD), first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neuropsychiatric disorder which begins with short term memory loss and proceeds to loss of cognitive functions, disorientation, impairment of judgement and reasoning and, ultimately, dementia. It is the most common form of dementia. The neuropathology is characterized by the formation in brain of amyloid plaques and neurofibrillary tangles. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. Moreover, as adults, born during the population boom of the 1940's and 1950's, approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Familial forms of AD are genetically heterogeneous, but most with early onset are linked to mutations in the presenilin genes *PSEN1* and *PSEN2*, as well as to mutations of the amyloid precursor gene *APP*. The majority of AD patients have no obvious family history and are classified as sporadic AD. For this late onset AD, several putative genetic risk factors have been reported. Among these the ApoE-epsilon 4 (ApoE 4) has been widely confirmed to confer increased risk for AD. Inheritance of ApoE4 and other risk factors are neither necessary nor sufficient to cause AD. In contrast to the APP- and PSEN mutations which increase the production of Aβ, the principal component of senile plaques in AD brain, the ApoE variant most likely influences Aβ accumulation by modulating clearance and degradation of the peptide.

In the search for biochemical changes in patients with neuropsychiatric and neurodegenerative disorders analysis of cerebrospinal fluid (CSF) may be a

useful method, since the CSF is continuous with the extracellular fluid of the brain. Therefore, a plurality of studies aiming at the analysis of the central nervous system (CNS) specific proteins in CSF were performed in order to find biochemical markers for neuronal and synaptic function and pathology in degenerative brain disorders.

Nerve growth factor (NGF) is one of the neurotrophic agents that promote differentiation or support the survival and functioning of some populations of neurons, influencing their effects not only on the peripheral sensory and sympathetic neurons but also on the central neurons. The pathophysiological role of NGF in the human nervous system, especially in relation to neuropsychiatric disorders, has not been fully understood yet. It is known that patients with acute multiple sclerosis (MS), traumatic brain injury or hypertensive cerebral hemorrhage show higher NGF levels in the CSF and NGF has trophic roles in regenerating axons in the CNS.

To determine the pathophysiological roles of NGF in the human CNS with special reference to neuropsychiatric disorders, levels of NGF in CSF from patients with the following neurodegenerative disorders have been examined by Nisho et al. (Clinica Chimica Acta 275, 93 – 98, 1998) using a highly sensitive two-site enzyme immunoassay:

(i) Parkinson's disease
(ii) Progressive supranuclear palsy
(iii) Sporadic olivo-ponto-cerebellar atrophy
(iv) Spinocerebellar ataxia 3 / Machado-Joseph disease
(v) Dentato-rubro-pallido-luysian atrophy

However, Nisho et al. did not examine any patients suffering from Alzheimer's disease.

Lappalainen et al. (Journal of Child Neurology 11 (4), 296 – 300, 1996) report about low levels of NGF in cerebrospinal fluid of children with Rett Syndrome.

Dicou et al. (Autoimmunity 26 (3), 189 – 194, 1997) report that no changes in anti-NGF autoantibody titers or in NGF frequency are detected in sera of AD patients, suggesting that they are not involved in the neuroimmunological mechanisms underlying AD.

Lorigados et al. (Journal of Neuroscience Research 32 (3), 329 – 339, 1992) applied a two-site enzyme immunoassay to examine NGF levels in normal human serum and serum from Alzheimer patients.

Massaro et al. (Italian Journal of Neurological Science 15 (2), 105 – 108, 1994) studied NGF in cerebrospinal fluid from patients with various neurological disorders including AD. Their study does not support the possibility that NGF is involved in the neuroimmunological mechanisms which can be expected to be linked in the inflammatory or degenerative diseases of the central and peripheral nervous system chosen in their study.

Crutcher et al. (The Journal of Neuroscience 13 (6), 2540 – 2550, 1993) used a two-site ELISA and a bioassay to detect NGF-like activity in human brain tissue. NGF-like activity was significantly elevated in the frontal and occipital cortex from patients with AD. Their results demonstrate the feasibility of detecting NGF-like activity in both fresh and postmortem human brain tissue.

The international patent application PCT/EP 91/01100 discloses a method for the qualitative and quantitative determination of a polypeptide or protein analyte present in a biological fluid or a solution. This method is exemplified by the determination of NGF.

Seiger et al. (Behavioural Brain Research 57 (2), 255 – 261, 1993) report on the clinical outcome of a first case of intracranial infusion of NGF to an AD patient. This therapeutic attempt is based on animal research showing that NGF stimulates central cholinergic neurons of the type known to be lost during the development of AD.

Hoffer et al. (Journal of Neural Transmission, Suppl. 49, 1 – 10, 1997) disclose treatment strategies based on transfer of genes, molecules, or cells to the central nervous system. Before degeneration has occurred, it may be possible to rescue "stressed" neurons, and stimulate terminal outgrowth using treatment with neurotrophic factors. Such approaches, with an emphasis on the NGF family of neurotrophins and their receptors, are reviewed.

Lapchak (Experimental Neurology 124, 16 – 20, 1993) provides in his review an overview of the importance of NGF as a neurotrophic factor for adult cholinergic neurons of the septohippocampal pathway. Information concerning the possible therapeutic use of NGF or small molecules that increase the expression of NGF to treat the cholinergic neurodegeneration that occurs in AD are provided.

Sofroniew (Alzheimer's Research 2 (1-2), 7 – 13, 1996) discloses data from pilot studies of NGF infusion into the CSF of patients with AD, peripheral administration of NGF, which suggest that achieving a method for site specific delivery of NGF in the CNS may be an important consideration in developing a treatment strategy.

Murase et al. (Biochemical and Biophysical Research Communications 193 (1), 198 – 203, 1993) disclose that NGF level is not decreased in the serum, brain-spinal fluid, hippocampus, or parietal cortex of individuals with AD.

As AD is a growing social and medical problem, there is a strong need for *ante mortem* methods of diagnosing or prognosing said disease in subjects as well as for methods of treatment.

In one aspect, the invention features a method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In a further aspect, the invention features a method of monitoring progression of Alzheimer's disease in a subject, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In still a further aspect, the invention features a method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of a subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

An increase of a level of nerve growth factor in cerebrospinal fluid from a subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject. In particular, a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject. Specifically, a level of NGF in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of AD in said subject. Said subject is preferably a human. NGF can e.g. be detected using an immunoassay, a bioassay or a binding assay (see e.g. Crutcher et al., The Journal of Neuroscience 13 (6), 2540 – 2550, 1993).

It is particularly preferred to further compare a level and/or activity of nerve growth factor with a level and/or activity of NGF in a series of samples taken from said subject over a period of time. Said subject might have received a treatment prior to one or more of said sample gatherings. Said level and/or said activity are preferably determined before and after said treatment.

In a further preferred embodiment, additionally a level, or an activity, or both said level and said activity, of a further neurotrophin, e.g. neurotrophin 3 (NT-3), is determined with the goal of diagnosing, prognosing, evaluating the risk of developing, evaluating a treatment of, or monitoring the progression of Alzheimer's disease. In particular, a level of neurotrophin 3 ≥ 15 pg/ml indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In another aspect, the invention features a kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

In particular, an increase of said level of NGF in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of AD in said subject. In particular, a level of nerve growth factor $\geq$ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject. Specifically, a level of NGF in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of AD in said subject.

Additionally, said kit preferably further comprises at least one reagent which selectively detects neurotrophin 3 (NT-3). Combined testing of NGF and a further neurotrophin, in particular NT-3, is a valuable tool in the diagnosis, prognosis, or risk evaluation of Alzheimer's disease (see example 3 and table 1). In particular, a level of neurotrophin 3 $\geq$ 15 pg/ml indicates a diagnosis, prognosis, or increased risk of Alzheimer's disease.

In another aspect, the invention features a method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

It might be further preferred to administer to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In still another aspect, the invention features the use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

In still another aspect, the invention features a composition for use as a medicament comprising (i) a first agent which directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In a further aspect, the invention features the use of a composition for the manufacture of a medicament for treating Alzheimer's disease, said composition comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin, and a further neurotrophin. In a preferred embodiment, said further neurotrophin is neurotrophin 3. It is preferred that said agents reduce the corresponding activity or level of NGF or NT-3, respectively.

The invention further features a method for identifying an agent that directly or indirectly affects an activity, or a level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or a level, or both said activity and level, of at least one of said substances before and after said contacting.

Figure 1 relates to example 1 and depicts that CSF levels of NGF are significantly elevated in the AD group, as compared to both the group consisting of patients with major depression (DE) as well as to the control group (CTR). Levels (pg/ml) are given in mean ± SEM. Asterisk (*, **) indicate

significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p < 0.001$; ** AD versus CTR, $p < 0.001$. NGF concentrations in CSF of the AD group amounted to 8.79 +/- 0.72 pg/ml (mean +/- SEM, range: 3.29 to 14.95, n = 23), compared to 4.07 +/- 0.50 pg/ml in the DE group (range: 2.42 to 9.54, n = 14), and 3.49 +/- 0.51 pg/ml in the CTR group (range: 0.00 to 4.64, n = 8), respectively. The alterations in patients suffering from AD may reflect disturbances in the trophic support of specific neuronal populations, such as the basal forebrain cholinergic system. There was no apparent correlation of CSF levels of NGF with ApoE genotype (or phenotype, respectively), age, duration of AD, MMS, NOSGER or MADRS scores.

Figure 2 relates to example 2 and depicts nerve growth factor levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p = 0.002$; ** AD versus CTR, $p = 0.000$, *** DE versus CTR, $p = 0.000$. CSF levels of NGF were significantly elevated in the AD group, as compared to both the DE and the CTR group. CSF levels of NGF were also significantly elevated in the DE group, as compared to the CTR group. NGF concentrations in CSF of the AD group amounted to 8.19 +/- 0.91 pg/ml (mean +/- SEM, range: 0.00 to 23.00, n = 40), compared to 4.26 +/- 0.97 pg/ml in the DE group (range: 0.00 to 23.00, n = 22), and 1.18 +/- 0.35 pg/ml in the CTR group (range: 0.00 to 7.20, n = 32), respectively.

Figure 3 relates to example 3 and depicts neurotrophin 3 (NT-3) levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects (CTR). CSF levels of NT-3 were determined to define a cut-off value to be used in the combined tests shown in table 1. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * DE versus AD, $p = 0.005$; ** DE versus CTR, $p = 0.000$; *** AD versus CTR, $p = 0.010$. CSF levels of NT-3 were significantly elevated in the DE group, as compared to

both the AD and the CTR group. CSF levels of NT-3 were slightly, but significantly, elevated in the AD group, as compared to the CTR group. NT-3 concentrations in CSF of the DE group were 25.8 +/- 4.3 pg/ml (mean +/- SEM, range: 0.0 to 87.0, n =23), compared to 14.0 +/- 1.6 pg/ml in the AD group (range: 0.0 to 41.0, n = 39), and 10.5 +/- 1.6 pg/ml in the CTR group (range: 0.0 to 67.0, n = 63), respectively.

Table 1 relates to examples 2 and 3. This table shows the diagnostic accuracy of spinal fluid measurements of NGF and NT-3 in Alzheimer's Disease and Major Depression in the Elderly. In NGF measurements, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using a cut-off value of $\geq$ 4.0 pg/ml NGF (total: n = 94, AD: n = 40, DE: n = 22, CTR: n = 32). The combined NGF/NT-3 test showed a considerable specificity for the diagnosis of AD (90.1 %), using cut-off values of $\geq$ 4 pg/ml NGF, and < 15 pg/ml NT-3, respectively (total: n = 57, AD: n = 24, DE: n = 18, CTR: n = 15). Testing either NGF levels or NGF and NT-3 levels with suitable cut-off criteria constitutes candidate tools for specific biochemical diagnosis of AD. Using the opposite cut-off criteria, the combination test significantly separated AD patients from elderly DE patients with a specificity of 89.7 %. Therefore, another potential use of this test is the biochemical differentiation between these two frequent disorders in the elderly.

Table 2 depicts the clinical characteristics and test scores of patients with Alzheimer's disease (AD), major depression (DE) and non-demented control subjects (CTR). Nerve growth factor (NGF), neurotrophin 3 (NT-3), MMS (Mini Mental State), MADRS (Montgomery Asberg Depression Rating Scale), ApoE (apolipoprotein E), n.d. (not determined).

EXAMPLE 1

In order to achieve a differential diagnosis, the study included not only patients with AD, but also such with major depression (DE). Diagnosis of

probable AD was made according to criteria of the National Institute of Neuropsychiatric and Communicative Disorders and Stroke-Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA; McKhann et al., Neurology 34, 939 - 944, 1984). Patients with major depression were diagnosed according to the ICD10 (F32.0x/1x, F33.0x/1x) and DSM-IIIR (296.20-22, 296.30-32) criteria. All patients were referred to the research ward from general practitioners, neurologists and psychiatrists for diagnostic purposes and screening for clinical trials. None of the patients was institutionalized. The group of healthy control subjects (CTR) consisted of patients that underwent lumbar puncture for orthopedic or neurologic diagnostic purposes and were shown to have normal CSF cell counts, total protein levels, and absence of signs of blood barrier dysfunction or cerebral IgG synthesis, as well as absence of any cerebral disorders.

AD, DE and CTR patients were carefully examined and received a thorough clinical work-up. Psychometric testing including the Mini Mental State (MMS; Folstein et al., J. Psychiatry Res. 12, 189 - 198, 1975), as a global screening instrument for dementia, and the Nurses' Observation Scale for Geriatric Patients (NOSGER; Spiegel et al., J. Am. Geriatr. Soc. 39(4), 339 - 347, 1991) as a functional measure of dementia severity. The patients with DE showed no cognitive disturbances in the clinical examinations and the Mini Mental State scores were within the normal range. Severity of depression was rated by using the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery et al., Br. J. Psychiatry, 134: 382 - 389, 1979). Apolipoprotein (ApoE) genotyping, or, if DNA was not available, ApoE phenotyping was included in the laboratory screening in the AD patients.

CSF was obtained for diagnostic purposes in the AD and DE patients in which no lumbar puncture had been previously done during the routine diagnostic work-up. Different CSF volumes were available for the analysis of the neurotrophin proteins. This fact explains the different sample sizes for the

individual measurements. All available CSF samples were used for the analyses.

The AD group was as follows: n = 23, 12 men, 11 women, mean age 63.9 +/- 13.2 SD years, range 39 – 86 years, MMS score: mean 18.6 +/- 5.6 SD.

The DE group was as follows: n = 14, 5 men, 9 women, mean age 68.2 +/- 13.6 SD years, range 47 – 86 years, MMS score: mean 28.1 +/- 0.9 SD.

The CTR group was as follows: n = 8, 5 men, 3 women, mean age 60.1 +/- 18.1 SD years, range 31 – 81 years.

AD and CTR patients were free of psychotropic medication. Patients with major depression were treated with various antidepressant drugs including serotonin reuptake inhibitors, reversible monoaminooxidase A inhibitors and tricyclics. Informed consent was taken from each patient and their caregivers before the investigation. The study was approved by the local ethics committee. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Within one week of dementia testing, CSF was obtained by lumbar puncture. To control for possible influences of a ventriculo-lumbar gradient, lumbar punctures were done between 7.30 and 8 a. m. before breakfast while patients were still lying flat. CSF samples were frozen on dry ice immediately upon withdrawal at the bedside in 0.5 ml aliquots and stored at – 85 °C until biochemical analysis.

CSF levels of NGF were measured by an ELISA as described recently (Weskamp et al., J. Neurochem. 48, 1779 – 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim) diluted in carbonate buffer pH 9.2 over night at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room

temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D-galactopyranoside was added and plates were incubated at 4 °C over night. The reaction was stopped after 1 h at RT and the flurorescent product was measured in the microtiter wells using a fluorometer (Labsystems Fluoroskan Ascent FL) (excitation wavelength: 355 nm; emission wavelength: 460 nm). The detection limit was 1.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 %.

ApoE genotyping was performed using INNO-LiPA ApoE, Innogenetics, Belgium. ApoE phenotyping was performed according to McDowell et al. (Clin. Chem. 35(10), 2070 – 2073, 1989). The use of the ApoE phenotype synonymous with the ApoE genotype in the statistical analyses seemed to be appropriate, since ApoE genotyping compared with protein phenotyping showed conflicting results in less than 2 % (Hansen et al., Clin. Chim. Acta, 224(2), 131 – 137, 1994).

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Correlation analyses were performed by multiple regression using CSF levels of neurotrophins as well as ApoE genotype (or phenotype, respectively), age, duration of the disease in AD, MMS, NOSGER and MADRS scores. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

EXAMPLE 2

The study described in example 1 has been extended to a wider panel of patients as described below in this example 2.

Diagnosis, clinical examination and treatment of patients as well as lumbar puncture were performed as described in example 1.

For NGF measurements, 94 spinal fluid samples were examined. The AD group (n = 40) consisted of 18 men and 22 women, mean age 68.8 +/- 12.4 SD years, range 39 - 88 yr, MMS score: mean 19.3 +/- 4.6 SD. The DE group (n = 22) consisted of 8 men and 12 women, mean age 69.8 +/- 12.6 SD years, range 47 - 86 yr, MMS score: mean 27.5 +/- 2.1 SD. CTR group: n = 32, 18 men, 14 women, mean age 64.0 +/- 14.9 SD years, range 29 - 96 yr.

CSF levels of NGF were measured by an ELISA as described by Weskamp et al. (J. Neurochem. 48: 1779 - 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim) diluted in carbonate buffer pH 9.2 overnight at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D- galactopyranoside was added and plates were incubated at 4 °C overnight. The reaction was stopped after 1h at RT, and the fluorescent product was measured in the microtiter wells by using a fluorometer (Labsystems Fluoroskan Ascent FL) at 355 nm excitation and 460 nm emission wavelength. The detection limit was 0.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 % and the assay was linear over a range of 0.5 to 500 pg/ml.

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

To estimate the diagnostic accuracy of the test, a) sensitivities and b) specificities, defined as follows, were calculated: a) true positives / (true

positives and false negatives), and b) true negatives / (true negatives and false positives). To estimate the probability of disease, predictive values of the tests were calculated. The positive predictive value (PPV) was defined as true positives / (true positives + false positives). The negative predictive value (NPV) was defined as true negatives / (true negatives + false negatives).

EXAMPLE 3

The purpose of this study was to check whether combined measurements of the CSF levels of NGF and neurotrophin-3 (NT-3) – which also belongs to the group of neurotrophins – improves the diagnostic accuracy of the NGF test described in example 2.

In a first step, NT-3 levels in CSF were determined to define a cut-off value to be used in the combined tests. Diagnosis, clinical examination and treatment of patients, lumbar puncture and statistical analyses were performed as described in example 2. For NT-3 measurements, 125 spinal fluid samples were examined. AD group: n = 39, 20 men, 19 women, mean age 67.2 +/- 11.5 SD years, range 39 – 86 yr, MMS score: mean 19.1 +/- 5.3 SD. DE group: n = 23, 8 men, 15 women, mean age 70.5 +/- 11.9 SD years, range 47 – 86 yr, MMS score: mean 27.2 +/- 2.5 SD. CTR group: n = 63, 35 men, 28 women, mean age 56.0 +/- 15.0 SD years, range 28 – 84 yr. NT-3 was determined by using commercially available ELISA systems (Promega, Madison, WI) according to the manufacturer's protocol. 120 µl of undiluted CSF in carbonate buffer (pH 9.7) were added to 96 well immunoplates (Nunc) at 4 °C overnight. Anti-Human-NT-3 polyclonal antibodies (pAb) were used as capture Ab. Anti-NT-3 mAb were used as reporter Ab. After incubation with a species-specific Ab (anti-rat IgG) conjugated to horseradish peroxidase (HRP) as a tertiary reactant, and washing, the solution was incubated with the chromogenic substrate TMB (3, 5, 3', 5'-tetramethylbenzidine). Absorbance

was measured at 450 nm by using a microplate reader (Dynatech MR 700). NT-3 ELISA: linear range 4.7 – 300 pg/ml; cross-reaction with other neurotrophins at 10 ng/ml < 3%; detection limit 6.0 pg/ml.

57 CSF samples were available for combined NGF/NT-3 measurements. AD group: n = 24, 13 men, 11 women, mean age 64.9 +/- 12.5 SD years, range 47 – 82 yr, MMS score: mean 18.6 +/- 5.4 SD. DE group: n = 18, 7 men, 11 women, mean age 69.5 +/- 12.7 SD years, range 47 – 84 yr, MMS score: mean 27.7 +/- 2.1 SD. CTR group: n = 15, 10 men, 5 women, mean age 59.0 +/- 15.9 SD years, range 29 – 80 yr.

Claims (amended)

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

2. A method of monitoring progression of Alzheimer's disease in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

3. Use of the method according to claims 1 or 2 for evaluating a treatment for Alzheimer's disease.

4. The method according to any of claims 1 to 3, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

5. The method according to claim 4, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. The method according to any of claims 1 to 5, wherein said subject is a human.

7. The method according to any of claims 1 to 6, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

8. The method according to any of claims 1 to 7, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

9. The method according to any of claims 1 to 8, wherein said subject receives a treatment prior to one or more of said sample gatherings.

10. The method according to any of claims 1 to 9, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

11. The method according to any of claims 1 to 10, further comprising:
determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;
wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status

indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

12. The method according to claim 11 wherein said neurotrophin is neurotrophin-3.

13. The method according to claim 12 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

14. A kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

15. The kit according to claim 14 wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

16. The kit according to claim 15 wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. The kit according to any of claims 14 to 16 further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status,

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

18. The kit according to claim 17 wherein said neurotrophin is neurotrophin-3.

19. The kit according to claim 18 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

20. The kit according to any of claims 14 to 19 for use in monitoring a progression of Alzheimer's disease in a subject.

21. The kit according to any of claims 14 to 19 for use in monitoring the success or failure of a therapeutic treatment of a subject.

22. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective

amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

23. Use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

24. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

25. A composition for use as a medicament comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin and a further neurotrophin.

26. A composition according to claim 25 wherein said further neurotrophin is neurotrophin-3.

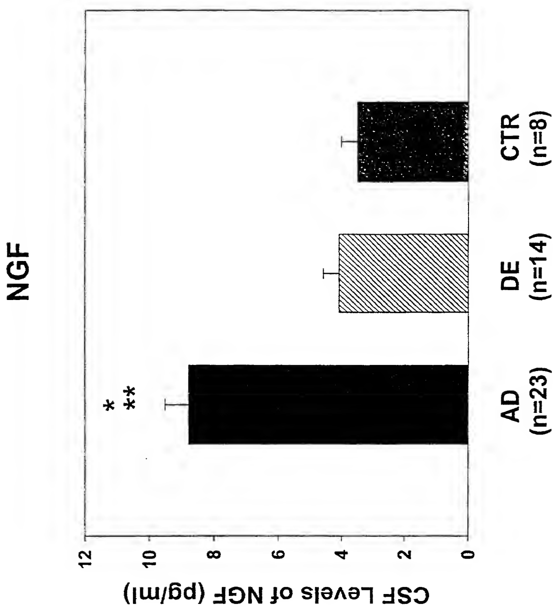

Fig. 1

Fig. 2

NT-3
CSF Levels of NT-3 (pg/ml)
35
30
25
20
15
10
5
0
***
**
*
AD (n=39)
DE (n=23)
CTR (n=63)

Fig. 3

Spinal Fluid Measurements of NGF and NT-3: Diagnostic Accuracy in Alzheimer's Disease (AD) and Major Depression in the Elderly (DE)

	NGF test (NGF>4 pg/ml)	**NT-3 test** (NT-3>15 pg/ml)
	Diagnosis of AD	**Diagnosis of DE**
Sensitivity	71,9%	73,9%
Specificity	79,2%	86,1%
Positive Predictive Value (PPV)	67,6%	50,0%
Negative Predictive Value (NPV)	82,4%	91,7%
	Combined NGF/NT-3 test (NGF>4 pg/ml, NT-3<15 pg/ml)	**Combined NGF/NT-3 test** (NT-3>15 pg/ml, NGF<4 pg/ml)
	Diagnosis of AD	**Diagnosis of DE**
Sensitivity	62.5%	55.5%
Specificity	90.1%	89.7%
Positive Predictive Value (PPV)	83.3%	71.4%
Negative Predictive Value (NPV)	76.9%	81.4%

Table 1

Clinical Characteristics

		AD	DE	CTR
NGF measurements	n	40 (18 m, 22 f)	21 (8 m, 13 f)	32 (18 m, 14 f)
	Age (mean±SD) yrs	68.8±12.4	69.3±12.6	64.0±14.9
	Range (yrs)	39-88	47-86	29-96
	MMS score (mean±SD)	19.3±4.6	27.6±2.1	n.d.
	MADRS score	n.d.	18.6±9.7	n.d.
	ApoE (%)	2/3 (14), 3/3 (36)	2/3 (23), 3/3 (34)	n.d.
		3/4 (43), 4/4 (7)	3/4 (31), 4/4 (12)	
NT-3 measurements	n	39 (20 m, 19 f)	n.d.	63 (35 m, 28 f)
	Age (mean±SD) yrs	67.2±11.5		56.0±15.0
	Range (yrs)	39-86		28-84
	MMS score (mean±SD)	19.1±5.3		n.d.
	MADRS score	n.d.		n.d.
	ApoE (%)	2/3 (14), 3/3 (34)		n.d.
		3/4 (45), 4/4 (7)		

Table 2

ALL PATENTS, INCLUDING DESIGN, FOR APPLICATION BASED ON PCT, PARIS CONVENTION, NON PRIORITY; OR PROVISIONAL APPLICATIONS

JAN 22 2002 PATENT & TRADEMARK OFFICE

DECLARATION AND POWER OF ATTORNEY U.S.A.

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO. 163 #4

101 As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

102 Methods of diagnosing or treating Alzheimer's disease on basis of increased cerebro-spinal fluid levels of nerve growth factor

which is described and claimed in:

- [X] PCT International Application No. PCT/EP 00/03913 filed May 2, 2000
- [ ] the attached specification
- [ ] the specification in application Serial No. _____ filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed Yes	No
99 108 722.2	EP	May 3, 1999	[X]	[ ]
99 120 211.0	EP	October 9, 1999	[X]	[ ]
			[ ]	[ ]

103 104 I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105 I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (42,772)

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*Inventor(s) name must include at least one unabbreviated first or middle name.

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	POST OFFICE ADDRESS: POST OFFICE ADDRESS: University of Basel Petersgraben 35	CITY: Basel	STATE OR COUNTRY: Swiss	ZIP CODE: 4003	

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
Uwe M. Mamm		
DATE NOVEMBER 20, 2001	DATE	DATE

[ ] Additional inventors are named on separately numbered sheets attached hereto.

001164 WO

DECLARATION AND POWER OF ATTORNEY U.S.A.

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO. 293 #4

ALL PATENTS, INCLUDING DESIGN FOR APPLICATION BASED ON PCT; PARIS CONVENTION; NON PRIORITY; OR PROVISIONAL APPLICATIONS

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or an additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

101 Methods of diagnosing or treating Alzheimer's disease on basis of increased cerebro-spinal fluid levels of nerve growth factor

102 which is described and claimed in:

- [X] PCT International Application No. PCT/EP 00/03913 filed May 2, 2000
- [ ] the attached specification
- [ ] the specification in application Serial No. _____ filed _____ (if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

103 Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed Yes	No
99 108 722.2	EP	May 3, 1999	[X]	[ ]
99 120 211.0	EP	October 9, 1999	[X]	[ ]
			[ ]	[ ]

104 I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105 I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,840); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772)

SEND CORRESPONDENCE TO: CUSTOMER NO. 00136 or JACOBSON HOLMAN PROFESSIONAL LIMITED LIABILITY COMPANY 400 SEVENTH STREET, N.W. WASHINGTON, D.C. 20004	DIRECT TELEPHONE CALLS TO: (please use Attorney's Docket No.) (202) 638-6666 JACOBSON HOLMAN PROFESSIONAL LIMITED LIABILITY COMPANY

*Inventor(s) name must include at least one unabbreviated first or middle name.

201	FULL NAME * OF INVENTOR	FAMILY NAME Nitsch	GIVEN NAME Roger	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Zollikon	STATE OR FOREIGN COUNTRY Swiss	COUNTRY OF CITIZENSHIP Germany	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS Guggerstr. 19	CITY Zollikon	STATE OR COUNTRY Swiss	ZIP CODE 8702
202	FULL NAME * OF INVENTOR	FAMILY NAME Hock	GIVEN NAME Christoph	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Zürich	STATE OR FOREIGN COUNTRY Swiss	COUNTRY OF CITIZENSHIP Germany CHX	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS University of Zürich Lengstr. 31	CITY Zürich	STATE OR COUNTRY Swiss	ZIP CODE 8029
203	FULL NAME * OF INVENTOR	FAMILY NAME Otten	GIVEN NAME Uwe	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY Basel	STATE OR FOREIGN COUNTRY Swiss	COUNTRY OF CITIZENSHIP Swiss	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS University of Basel Petersgraben 35	CITY Basel	STATE OR COUNTRY Swiss	ZIP CODE 4003

2-0

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
DATE	DATE Nov. 20, 2001	DATE

[ ] Additional inventors are named on separately numbered sheets attached hereto.

00/154 WO

#4

DECLARATION AND POWER OF ATTORNEY U.S.A.

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO. 3 of 3

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

101 Methods of diagnosing or treating Alzheimer's disease on basis of increased cerebro-spinal fluid levels of nerve growth factor

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[X] PCT International Application No. PCT/EP 00/03913 filed May 2, 2000

[ ] the attached specification

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(if applicable) and amended on _____

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Prior Foreign Application(s)			Priority Claimed
99 108 722.2 (Number)	EP (Country)	May 3, 1999 (Day/Month/Year Filed)	[X] Yes [ ] No
99 120 211.0 (Number)	EP (Country)	October 9, 1999 (Day/Month/Year Filed)	[X] Yes [ ] No
103 (Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes [ ] No

104 I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105 I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

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JACOBSON HOLMAN PROFESSIONAL LIMITED LIABILITY COMPANY 400 SEVENTH STREET, N.W. WASHINGTON, D.C. 20004	**JACOBSON HOLMAN** PROFESSIONAL LIMITED LIABILITY COMPANY

*Inventor(s) name must include at least one unabbreviated first or middle name.

	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
201		Nitsch	Roger		
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
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202	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
		Hock	Christoph		
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
		Zürich	Swiss	Germany	
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3-20	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
		Otten	Uwe		
203	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
		Basel	Swiss	Swiss CH	
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
		University of Basel Petersgraben 35	Basel	Swiss	4003

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
DATE	DATE	DATE Basel Nov. 20, 2001

[ ] Additional inventors are named on separately numbered sheets attached hereto.